Malaria Vaccine Research at the Naval Medical Research Center

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At a time in bioscience when many people assume that vaccines are available for most infectious diseases, it often comes as a surprise that the ancient disease, malaria, is still the most important parasitic infectious disease in the world today. It also poses one of the greatest threats to the U.S. military operational forces than any other naturally-occurring infectious disease. In fact, in every campaign this century fought where malaria was present, more casualties resulted from malaria than from bullets. During the war in Vietnam, entire divisions were rendered ineffective due to large numbers of malaria cases.(*1*) Even more importantly for the world population there are between 300 and 500 million cases each year and between 1.5 and 2.7 million deaths annually, mostly in children living in Sub Saharan Africa.(*2*)

Drugs used to prevent malaria infection, though universally effective until the 1960's and 1970's, are either no longer effective or are becoming less effective in many parts of the world due to the development of drug resistance. And yet despite over 15 years of research, there is still no licensed vaccine against malaria.

Navy malaria researchers are developing approaches to preventing and treating malaria through drug and vaccine development at the Naval Medical Research Center (NMRC) core Malaria Program located in Silver Spring, MD, and also through a network of overseas research commands—Naval Medical Research Units (NMRU) working throughout Southeast Asia (NMRU-2), South America (NMRC-DET), Africa (NMRU-3) and most recently in Ghana, West Africa. In addition, NMRC has established the first Navy Clinical Trials Center at the National Naval Medical Center, Bethesda, MD, the only clinical trials center dedicated for malaria vaccine testing and development.

Malaria Life Cycle

So why is there no vaccine against a disease that causes such an incalculable costs in terms of human lives and suffering and results in the death of more than a million children each year? Many feel that the answer lies in the complexity of the parasite itself. Unlike most viruses and bacteria, pathogens such as malaria live a complex life. The single cell parasite *Plasmodium* starts its life in humans as "sporozoite" forms injected by the bite of an infected female *Anopheles* mosquito. Hundreds of sporozoites enter the blood stream with the mosquito's saliva and within minutes, invade individual cells in the liver. Inside these liver cells the parasites multiply to over 10,000 in 1 week.

As the parasites at this stage appear to cause no signs of illness, many feel this is an ideal time to attack the parasite with drugs and vaccines. If left unchecked, the parasites (now called "merozoites" and numbering over one million) burst out of the liver cells into the blood stream and attach themselves to red blood cells.

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Individual parasites enter the red blood cells and multiply to 16-20 parasites in approximately 2 days. All manifestations of disease occur at this stage of the parasite's life cycle.

Approximately, every 2 days parasites burst from the infected red blood cells, invade new red blood cells, and multiply an additional 10-20 times. Within a few days the numbers of parasites in the body reach many billions and cause fevers, chills, fatigue, and lethargy. In severe cases, especially in children, the parasites destroy so many red cells and suppress the production of new red cells that severe anemia develops. In some cases, the infected red cells become "sticky" and attach to the inner surface of capillaries, especially in the major organs, causing blockages.

When the parasites block the blood flow in the brain "cerebral malaria" develops resulting in seizures and coma. These two syndromes, severe anemia or cerebral malaria, are the predominant causes of death in children and non-immune travelers such as military personnel.

In order to reproduce, some parasites in the red cells do not divide and multiply, but transform into male and female "gametocytes." These gametocytes are taken up by feeding mosquitoes and sexually reproduce in the mosquito's stomach. The resulting sporozoites travel to the mosquito salivary glands ready to be injected on the next blood meal and to infect another individual.

Malaria Vaccine Development

Recognizing the complexity of the parasite, many scientists feel that an effective vaccine may need to attack multiple stages of the parasite's life cycle. In addition, the vaccine will have to mount different immune responses to attack the parasite at its different stages. For example, soluble circulating antibodies may be needed to attack the sporozoites while in the blood stream, killer immune cells (T-cells) may be needed to attack the parasites within the liver cells, and a combination of antibodies and T-cells may be required to attack the parasites inside the red cells. Further, antibodies may be needed to prevent the reproduction of the parasites within the mosquito stomach.

No vaccine to date has been shown to provide the breadth of immune responses that may be required for preventing malaria. The challenge is daunting, though there are two models that suggest that a protective malaria vaccine is feasible. If *Plasmodium* infected mosquitoes are bombarded with a precise amount of gamma irradiation, the parasites living inside the mosquito salivary glands become weakened but are not killed. When the mosquitoes are allowed to feed on an animal or human, the "irradiated sporozoites" invade liver cells normally, but they do not divide and do not develop further. The immune system recognizes the parasite proteins present in the liver cells and mounts an attack on these cells killing the parasite. This "irradiated sporozoite vaccine" is comprised of allowing 150 mosquitoes to feed on the arm of an individual once a month for 6 months. Individuals immunized by this method are completely protected against infection with malaria for at least 9 months. Unfortunately, this effective vaccine is completely impractical except as an experimental model.

A second vaccine model is that of "naturally acquired" immunity. Children raised in malarious areas who are continually exposed to the bites of infected mosquitoes and who survive to the age of about 10 years old, generally develop immunity to malaria. This immunity does not result in sterile protection, but does protect against severe disease. In fact, in many areas of the world, it is not uncommon to find nearly all adolescents and adults with malaria parasites in their blood yet they are apparently quite healthy. In these individuals the immune system keeps the number of parasites in the blood low and prevents the manifestations of severe disease. Interestingly, when these individuals travel to non-malarious areas, they often lose a substantial part of their immunity and are at risk for more severe malaria when they return.

Armed with these two models of malaria vaccines, it is convenient to approach vaccine development in one of three ways. The first is to assume that there are one or two "key" proteins that the parasite expresses and can be targeted for vaccine development. One would construct a vaccine that produces a very strong immune response against these one or two proteins and hope that this will be sufficient for killing the malaria parasite. For example, one protein expressed at the liver stage and one protein expressed at the blood stage could be targeted. In fact, the best experimental malaria vaccine to date is based on a single protein expressed at the sporozoite and liver stages. This vaccine, RTS,S, is a recombinant protein vaccine (a protein produced in the laboratory that is similar to a protein actually made by the parasite) and has been shown to provide 30 percent protection for several weeks in studies.(*3*) It is hoped that this vaccine can be improved upon sufficiently, perhaps by adding additional recombinant proteins, to provide long-term protection.

However, it may be that the parasite is sufficiently complex and has the ability to evade the host's immune

system as to require an attack at many targets simultaneously. This second approach targets all known proteins (10 to 15) expressed at multiple stages of the parasite life cycle. Unfortunately, it may not be practical to produce the multiple recombinant proteins required due to their expense, complexity of production and difficulty in purification.

To develop this type of multistage vaccine, a nontraditional approach may be needed. Researchers at the Malaria Program, Naval Medical Research Center, have been developing novel DNA-based vaccines used alone or in combination with recombinant proteins and viruses to target multiple stages of the parasite life cycle as well as exploiting the two models of immunity to malaria, irradiated sporozoites and naturally-acquired immunity. A third approach would target all expressed proteins from a particular stage, in essence reproducing the "whole organism" immunity seen in both the irradiated sporozoite vaccine and the naturally-acquired immunity models. To do this would require the identification and pattern of expression of all known genes and proteins from each of the stages of the parasite that are the targets of these vaccines, as well knowing which are those that generate protective immune responses, an approach that is actively being developed at the Navy's malaria program.

DNA-based vaccines

DNA vaccines are different from any other licensed vaccine available. Instead of immunizing with a foreign protein, DNA vaccines immunize with the genetic blueprint that encodes for that foreign protein. Cells from immunized individuals take up a "ring of DNA" containing the foreign gene and uses its own cellular machinery to translate the injected genetic material to produce the foreign protein. The immune system then responds to the foreign protein much in the same way that it would if the protein was introduced intact.

Thus DNA vaccines offer significant advantages over other types of vaccines. They are relatively easy to produce and purify, and thus are easy to modify; stimulate strong T-cell responses; may not require refrigeration; and have the potential for combination allowing the targeting of multiple foreign proteins simultaneously. During the past 6 years the NMRC Malaria Program has developed extensive experience with DNA-based vaccines demonstrating that DNA vaccines can protect mice from infection with malaria,(*4*) can be combined to overcome the differences in responses between different strains of mice,(*5*) can be combined with recombinant protein or recombinant pox viruses to increase protection in mice,(*6*) are immunogenic in monkeys(*7*) and can protect monkeys from lethal infection with malaria (Rogers, et al, submitted).

Based on these findings and others, NMRC's Malaria Program, conducted the first-ever clinical trial in healthy humans using a DNA vaccine.(*8*) This vaccine, though not designed to be protective, was shown to be safe, well tolerated and to produce strong cellular immunity.(*9*) A second trial evaluated this same DNA vaccine in a novel vaccine delivery system, the needle-less Biojector system (Epstein/Wang, et al submitted).

To assess the safety and immune responses to multiple genes introduced simultaneously a clinical trial was conducted using a combination of five malaria genes, targeted to the liver stage of the parasite life cycle. During the development of this five-gene vaccine, researchers at NMRC's Malaria Program, demonstrated that if these malaria genes were reengineered in the laboratory each had the potential to function significantly better than the original native malaria genes. Since *Plasmodium* DNA uses a very different balance in its genetic code as compared to humans, by changing the genetic code in the malaria genes to be more like that of human genes, they were able to show these genes could produce 10-20 times more foreign protein than the native malaria genes.

In studies in mice, these "synthetic genes" stimulated an immune response in mice 3-20 times greater than the native genes. Clinical trials are now being planned to assess the effectiveness of these synthetic vaccines.

Although DNA-based vaccines hold tremendous promise, not only for malaria vaccines, but for a variety of other diseases, based on results to date, they may not be able to simulate sufficiently strong immune responses in monkeys and humans on their own to confer complete protection against malaria. Researchers at NMRC and elsewhere have shown that combining a DNA vaccine "prime" followed with the same genes expressed by a modified virus, similar to the smallpox vaccine, or to recombinant protein, provides better immune responses and protection in animal models than either alone.(*10-12*)

A major effort, therefore, is underway focusing on improving malaria vaccine development by this "prime-boost" approach.. Because DNA vaccines on their own offer such a tremendous potential advantage over all other types of vaccines available today, a major area of research is focusing on improvements in vaccine delivery systems to make DNA vaccines on their own as effective

as the prime-boost vaccines. This includes novel means of getting the DNA into the cells, using absorbable micro particles, coated gold-beads, specialized polymers, and others; improving the DNA sequences in the vaccines used by the host cell machinery; and adding additional DNA vaccines expressing proteins helpful in recruiting immune cells to the area of vaccine delivery.

Genomics

The future of vaccine development for malaria and a range of other pathogens may actually lie within the genetic code itself. In order to duplicate whole organism immunity, such as occurs in both the irradiated sporozoite model and the naturally acquired immunity model, it may be necessary for the immune system to mount multiple types of immune responses simultaneously against multiple targets. Herein lies that power of the genetic code.

In 1996, Malaria Program researchers in an international collaboration with The Institute for Genomic Research (TIGR), the Sanger Centre, and Stanford University, with funding from the Department of Defense, the NIH, the Burroughs Wellcome Fund, and the Wellcome Trust, embarked on a project to determine the entire genetic sequence of the human malaria parasite, *P. falciparum.*(*13*) With the complete genomic sequence of the malaria parasite in hand, researchers would have the ability to identify every potential drug and vaccine target, elucidate complex biochemical pathways, and be able to develop tools to study fundamental parasite biological processes.

Within 18 months of starting the project, NMRC/ TIGR researchers published the first complete genetic sequence of a malaria parasite chromosome.(*14*) A second chromosome was published shortly after.(*15*) Plans are now underway to publish a series of articles by early 2002 including the complete genomic sequence of *P. falciparum.*

As part of the DNA sequencing consortium's effort, all of the genomic sequence data has been released to the public during the 4-year history of the project. This early released data has enabled researchers to "jump start" their research already leading to the identification of two potentially important targets for new antimalarial drugs.(*16,17*) The consortium has made such progress with the completion of the *P. falciparum* project, including overcoming several technical hurdles as well as developing improved and more cost effective means of sequencing, that the genomes from at least two additional species of malaria parasite will be completed by the end of 2002, including the second most important malaria parasite in humans, *P. vivax.*

Functional Genomics

NMRC researchers have also been on the forefront in developing and utilizing novel technologies to exploit the enormous amounts of data generated from the malaria genome project toward drug and vaccine development. Working broadly on several fronts, the NMRC team has produced the first chromosome-specific DNA microarray, enabling the study of the expression of thousands of genes simultaneously, for example in response to antimalarial drugs and from various stages of the parasite life cycle.

In collaboration with Scripps Research Institute (SRI) they have employed a method to identify hundreds to thousands of parasite proteins from various stages of the life cycle using high-throughput liquid chromatography coupled with tandem mass spectrometry, have partnered with San Diego Supercomputing Center and the U.S. Navy High Performance Computing Center (supercomputers) to dramatically improve computer performance used in protein identification, deployed recombinational cloning systems to produce hundreds of plasmid clones in a fraction of the time by traditional methods, and developed in-house relational database capabilities needed to handle the enormous volumes of information generated from these functional genomics projects.(*18*)

DNA-based vaccines offer a unique opportunity to transform genomic sequence data into deployable vaccines. Although "whole genes" are currently being investigated as potential DNA vaccines, researchers at the NMRC malaria program are also constructing "minigene" vaccines, comprised of a series of "epitopes," small regions of individual genes predicted to be important in stimulating immune responses, particularly T-cell responses.(*19-21*) The next generation of DNA-based vaccines that will result from the data from the Malaria Genome Project will likely be composed of dozens to hundreds of epitopes strung together like beads on a string.

Conclusion

By the end of 2002, researchers will have at their disposal the complete genomic sequence of *P. falciparum*, and its two hosts: humans(*22*) and the mosquito, *Anopheles gambiae.* In this nascent field of

genomics, it is as yet unclear how these data will be best used to elucidate the complex interactions between the parasite and its host(s). However, combined with technical advances in functional genomics and rational drug and vaccine design, including DNA-based vaccines, these genomic data may provide the foundation for entirely novel approaches to drug and vaccine development, and may lead to new strategies to combat not only malaria but many other infectious and emerging diseases. The challenge over the next decade will be to use these novel approaches to better understand the malaria parasite and its interactions with its hosts, to develop strategies to intervene and reduce the tremendous human toll, and to provide effective vaccines and drugs to protect DOD forces against the threat of this destructive disease. Navy researchers are at the cutting edge in this 21st century era of biomedicine.

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